REMARKS

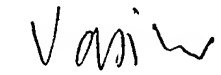
This Preliminary Amendment is being filed concurrently with a Response to Notice to Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Disclosures.

The Examiner noted that the claims and pages 11 and 21 of the specification lacked SEQ ID designation. The specification has been amended to reflect the Examiner's comments. The forward primer sequence on p. 21 of the specification has been amended to change a typographical error: in the amended version of the specification, the non-existing designation of nucleic bases ("f" and "l") has been replaced with the correct nucleic bases ("t" and "t").

The specification has been further amended to incorporate the hard copy of the Sequence Listing and the corresponding computer-readable form, both of which are submitted herewith. This Preliminary Amendment does not add new matter and does not reflect a departure therefrom in substance or variation in the disclosure of U.S. Patent Application 10/806,915 filed on March 23, 2004.

Dated: October 30, 2006

Respectfully submitted,



Vyacheslav Vasilyev
Attorney for Applicant(s)
Registration No. 58,154

Fox Rothschild LLP
997 Lenox Drive, Bldg. #3
Lawrenceville, NJ 08648
Telephone: (609) 844-3021
Facsimile: (609) 896-1469

[032] The nucleotide sequence encoding the LIM mineralization protein preferably hybridizes under standard conditions to a nucleic acid molecule complementary to the full length of the following sequence:

tcctcatccg ggtcttgcat gaactcggtg (SEQ. ID. NO. 9)

or hybridizes under highly stringent conditions to a nucleic acid molecule complementary to the full length of the following sequence:

gcccccgccc gctgacagcg ccccgcaa (SEQ. ID. NO. 10),

or both.

[033] "Standard hybridization conditions" will vary with the size of the probe, the background and the concentration of the nucleic acid reagents, as well as the type of hybridization (in *situ,* Southern blot, or hybrization of DNA-RNA hybrids (Northern blot)). The determination of "standard hybridization conditions" is within the level of skill in the art. Methods include, for example, those described in U.S. Patent 5,580,775 (Fremeau, *et al.),* Southern, J. Mol. Biol., 98:503 (1975), Alwine, *et al.,* Meth. Enzymol., 68:220 (1979), and Sambrook, *et al.,* Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Press, 7.19-7.50 (1989).

[034] One set of standard hybridization conditions involves pre-hybridizing a blot at 42°C for 2 hours in 50% formamide, 5X SSPE (150 nM NaCl, 10 mM Na H_2PO_4 [pH 7.4], 1 mM EDTA [pH 8.0]) 5X Denhardt's solution (20 mg Ficoll, 20 mg polyvinylpyrrolidone and 20 mg BSA per 100 ml water), 10% dextran sulphate, 1% SDS and 100 µg/ml salmon sperm DNA. A ^{32}P- labeled cDNA probe is added, and further hybridizing continued for 14 hours. Afterward, the blot is washed twice with 2X SSPE, 0.1 % SDS for 20 minutes at 22°C, followed by a 1 hour wash at 65°C in 0.1 X SSPE, 0.1 %SDS. The blot is then dried and exposed to x-ray film for 5 days in the presence of an intensifying screen.

[056] The present invention may be more fully understood by reference to the following non-limiting examples.

Examples

[057] The synthesis and use of a (His)6 TAT-LMP protein conjugate, comprising the protein transduction domain of HIV-Tat and the LMP-1 protein, is described below. The pTAT-HA-vector was obtained under a material transfer agreement from Washington University (St. Louis, MO).

[058] An NcoI restriction site was added to the 5' end of hLMP-1 by utilizing 10 pCDNA3.1/hLMP-i as the template for PCR with the following primers:

Fwd: 5'-CCATGGA~~t~~TTCC~~t~~TTCAAAGTAGTGC-3' (SEQ. ID. NO. 11)

Rev: 5 `-CAGGGCGGGCGGCTGGTAG-3' (SEQ. ID. NO. 12)

The reaction was performed at: 95°C for 2 Min.[95°C, 30 sec; 66°C, 30 sec; 72°C, 1 mm] x 25, and 72°C 10 mm. The PCR product was cloned into PCRII-TOPO vector (Invitrogen) and appropriate clones were identified by sequencing.

[059] Construction of the (His)6 TAT-LMP vector was accomplished by restriction endonuclease digestion of plasmid clones with Ncol and Clal, and purification of the resulting product by agarose gel electrophoresis and electroelution. The full-length hLMP-1 sequence was isolated by restriction digest of the pCDNA3.1/hLMP-1 vector with Clal and EcoRI, and purification of the resulting product by agarose gel electrophoresis and electroelution. The pTAT-HA-vector was also subjected to restriction digestion with NcoI and EcoRl, and the resulting linearized vector was purified by agarose gel electrophoresis and electroelution. Products were then ligated by standard procedures overnight at 16°C. Correctly ligated products (5' hLMP-1 + 3' hLMP-1 + linearized pTAT-HA-vector = (His)6 TAT-

[032] The nucleotide sequence encoding the LIM mineralization protein preferably hybridizes under standard conditions to a nucleic acid molecule complementary to the full length of the following sequence:

tcctcatccg ggtcttgcat gaactcggtg (SEQ. ID. NO. 9)

or hybridizes under highly stringent conditions to a nucleic acid molecule complementary to the full length of the following sequence:

gcccccgccc gctgacagcg ccccgcaa (SEQ. ID. NO. 10),

or both.

[033] "Standard hybridization conditions" will vary with the size of the probe, the background and the concentration of the nucleic acid reagents, as well as the type of hybridization (in *situ,* Southern blot, or hybrization of DNA-RNA hybrids (Northern blot)). The determination of "standard hybridization conditions" is within the level of skill in the art. Methods include, for example, those described in U.S. Patent 5,580,775 (Fremeau, *et al.),* Southern, J. Mol. Biol., 98:503 (1975), Alwine, *et al.,* Meth. Enzymol., 68:220 (1979), and Sambrook, *et al.,* Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Press, 7.19-7.50 (1989).

[034] One set of standard hybridization conditions involves pre-hybridizing a blot at 42°C for 2 hours in 50% formamide, 5X SSPE (150 nM NaCl, 10 mM Na H_2PO_4 [pH 7.4], 1 mM EDTA [pH 8.0]) 5X Denhardt's solution (20 mg Ficoll, 20 mg polyvinylpyrrolidone and 20 mg BSA per 100 ml water), 10% dextran sulphate, 1% SDS and 100 µg/ml salmon sperm DNA. A ^{32}P- labeled cDNA probe is added, and further hybridizing continued for 14 hours. Afterward, the blot is washed twice with 2X SSPE, 0.1 % SDS for 20 minutes at 22°C, followed by a 1 hour wash at 65°C in 0.1 X SSPE, 0.1 %SDS. The blot is then dried and exposed to x-ray film for 5 days in the presence of an intensifying screen.

[056] The present invention may be more fully understood by reference to the following non-limiting examples.

Examples

[057] The synthesis and use of a (His)6 TAT-LMP protein conjugate, comprising the protein transduction domain of HIV-Tat and the LMP-1 protein, is described below. The pTAT-HA-vector was obtained under a material transfer agreement from Washington University (St. Louis, MO).

[058] An NcoI restriction site was added to the 5' end of hLMP-1 by utilizing 10 pCDNA3.1/hLMP-i as the template for PCR with the following primers:

Fwd: 5'-CCATGGATTCCTTCAAAGTAGTGC-3' (SEQ. ID. NO. 11)

Rev: 5 `-CAGGGCGGGCGGCTGGTAG-3' (SEQ. ID. NO. 12)

The reaction was performed at: 95°C for 2 Min.[95°C, 30 sec; 66°C, 30 sec; 72°C, 1 mm] x 25, and 72°C 10 mm. The PCR product was cloned into PCRII-TOPO vector (Invitrogen) and appropriate clones were identified by sequencing.

[059] Construction of the (His)6 TAT-LMP vector was accomplished by restriction endonuclease digestion of plasmid clones with Ncol and Clal, and purification of the resulting product by agarose gel electrophoresis and electroelution. The full-length hLMP-1 sequence was isolated by restriction digest of the pCDNA3.1/hLMP-1 vector with Clal and EcoRI, and purification of the resulting product by agarose gel electrophoresis and electroelution. The pTAT-HA-vector was also subjected to restriction digestion with NcoI and EcoRl, and the resulting linearized vector was purified by agarose gel electrophoresis and electroelution. Products were then ligated by standard procedures overnight at 16°C. Correctly ligated products (5' hLMP-1 + 3' hLMP-1 + linearized pTAT-HA-vector = (His)6 TAT-

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Titus et al.

Application No.: 10/806,915 Group Art Unit: 1636

Filed: March 23, 2004 Examiner: QIAN, Celine X.

For: INTRACELLULAR DELIVERY OF OSTEOINDUCTIVE PROTEINS AND PEPTIDES

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT THAT SUBSTITUTE SPECIFICATION CONTAINS NO NEW MATTER (37 C.F.R. §1.125)

1. Identification of person making this statement:

   Vyacheslav Vasilyev
   Fox Rothschild LLP
   997 Lenox Drive, Bldg. #3
   Lawrenceville, NJ 08648

2. The person making this statement is the registered patent attorney in this application, Registration Number 58,154.
3. I hereby state that the accompanying substitute specification sheets contain no new subject matter over that contained in the above-identified application originally filed.
4. I further state that the changes are the same as indicated in the interlineated original specification also accompanying this declaration.

Dated: October 30, 2006

Respectfully submitted,

Vasilyev

Vyacheslav Vasilyev
Attorney for Applicant(s)
Registration No. 58,154